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An ecological study of *Bithynia* snails, the first intermediate host of *Opisthorchis viverrini* in northeast Thailand

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ABSTRACT

Infection with the food-borne trematodiasis, liver fluke *Opisthorchis viverrini*, is a major public health concern in Southeast Asia. While epidemiology and parasitic incidence in humans are well studied, ecological information on the *O. viverrini* intermediate hosts remains limited. This study aimed to investigate the factors affecting the distribution and abundance of the first intermediate host, *Bithynia siamensis goniomphalos* snails. Water quality and snails were sampled in 31 sites in Muang District, Khon Kaen Province, Thailand from June 2012 to January 2013 to characterize the *B.s. goniomphalos* snail habitats. Species relative abundance and Shannon's diversity and evenness indices were employed to describe snail compositions and diversities across different habitat types. Statistical analyses were conducted to examine the extent to which the water quality variables and species interactions account for the relative abundance of *B.s. goniomphalos* snails. The results showed that the freshwater habitats of ponds, streams and rice paddies possessed significantly different abiotic water qualities, with water temperature and pH showing distinct statistical differences ($P < 0.05$). Different habitats had different snail diversity and species evenness, with high *B.s. goniomphalos* snail abundance at rice paddy habitats. The differences in snail abundance might be due to the distinct sets of abiotic water qualities associated with each habitat types. The relative abundance of *B.s. goniomphalos* snails was found to be negatively correlated with that of *Filopaludina martensi martensi* snails ($r = -0.46$, $P < 0.05$), underscoring the possible influence of species interaction on *B.s. goniomphalos* snail population. Field work observations revealed that rice planting seasons and irrigation could regulate snail population dynamics at rice paddy habitats. This study provides new ecological insights into the factors affecting *Bithynia* snail distribution and abundance. It bridges the knowledge gap in *O. viverrini* disease ecology and highlights the potential effect of anthropogenic irrigation practices on *B.s. goniomphalos* snail ecology.

1. Introduction

Food-borne parasitic infections are a major group of neglected diseases with more than 750 million people (>10% of the world's population) at risk (Keiser and Utzinger, 2009; Sripa, 2012). The infections are still endemic in many parts of the world where poverty persists, especially in Southeast Asia. The most important of these parasites endemic in the Lower Mekong region is *Opisthorchis viverrini*, a liver fluke that has been identified by the International Agency for Research on Cancer as a cause of cholangiocarcinoma (CCA), a fatal bile duct cancer (IARC, 1994). The life cycle of *O. viverrini* is complex, with snails of the genus *Bithynia* as the first intermediate hosts, Cyprinid fish as the second intermediate hosts, and humans and fish-eating carnivores, such as cats and dogs, as definitive hosts (Wykoff et al., 1965). Infected fish are the direct route by which *O. viverrini* is transmitted to human, upon consumption. Human infection is common in the Lower Mekong region because consumption of raw, fermented or inadequately cooked fish dishes is a traditional part of the diet (Sithithaworn et al., 2012; Wang et al., 2013). Although the life cycle of *O. viverrini* has been known for many decades, the bulk of research to date has focused on collecting epidemiological data from humans. Very little information is available on the ecology of the *O. viverrini* intermediate hosts. Because the first intermediate snail hosts have highly variable infection rates compared to the second intermediate fish hosts, *Bithynia* snails are likely to be the key link in the *O. viverrini*

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life cycle (Petney et al., 2012). This prompts the need for ecological investigations of the factors affecting *Bithynia* snail distribution and abundance.

The distribution and abundance of species depend on both abiotic and biotic factors (MacDonald, 2002). Within freshwater environments, physical and chemical properties of water, such as temperature, oxygen, salinity and acidity, are key abiotic modulators that can affect organisms' ability to survive (Dodds and Whiles, 2010; Chapin et al., 2011). For *Bithynia* snails, types of water bodies and water quality have been suggested as important determinants influencing snail distributions (Wang, 2012). *Bithynia* snails have been found in water environments with lower pH in northern Thailand (Ngern-klun et al., 2006). Conversely, salinity has been suggested to be the determinant for broad-scale distribution of *Bithynia siamensis goniomphalos* in northeast Thailand (Suwannatrai et al., 2011). While abiotic factors influence where a particular species is able to live, biotic factors such as intra-species and inter-species interactions for limited resources, often determine the species' success, thereby affecting species abundance. Indeed, species diversity and interaction have been extensively studied for Lyme disease and other helminthic diseases (Ostfeld, 2009; Keesing et al., 2010). *O. viverrini* literature regarding snail diversity and the correlations between different snail species collected from various freshwater environments is, however, still limited, with the exception of Haruay et al. (2008), in which a negative correlation between the abundance of *Filopaludina martensi martensi* snails and that of *B.s. goniomphalos* snails was identified. Nevertheless, the work can be further improved through standardized protocols of snail collections for quantification of biodiversity.

The objectives of this study are thus to examine the abiotic and biotic factors that affect the distribution and abundance of the first intermediate *O. viverrini* host, *Bithynia* snails. Specifically, this study addresses the following three research questions. First, do different freshwater habitats of *Bithynia* snails possess different water qualities? Second, do snail species compositions and diversities vary across different freshwater habitats, and do *Bithynia* snails dominate certain habitat type? Third, how do water quality and the presence of the snail species, *F.m. martensi* snails, affect *Bithynia* snail abundance? Understanding the characteristics of *Bithynia* snail habitats and the factors affecting *Bithynia* snail abundance will provide insights into *O. viverrini* disease ecology.

2. Materials and methods

2.1. Study area and sampling periods

The study area is in Khon Kaen Province, northeast Thailand (Fig. 1) where high human *O. viverrini* prevalence and opisthorchiasis-associated CCA have been reported (Sriamporn et al., 2004; Sithithaworn et al., 2012). The province, located in the Chi River catchment, has a tropical monsoon climate with distinctive dry and wet seasons. The dry season occurs between November and March; the rainy season is from May to October, with the wetter period occurring between August and September. Average annual rainfall is 1379.1 mm. With approximately 80% of the rain experienced during the rainy season, extensive flooding is often encountered toward the end of the rainy season. Mean minimum and maximum temperatures are 16.6 °C and 35.9 °C, respectively (TMD, 2012). In Thailand, different taxa of *Bithynia* snails have their almost exclusive geographic distributions; the species found in northeast Thailand is *Bithynia siamensis goniomphalos* (Sithithaworn et al., 2007).

Sampling sites were selected within the province in consideration of the potential *Bithynia* habitats and site accessibility. The study area consisted of three main *Bithynia* snail habitat types: ponds, streams and rice paddies. A total of 31 sampling sites in Muang District feasible for sampling were identified along the edges of the three habitat types, including 10 sites for ponds, 10 sites for streams and 11 sites for rice paddies. Of the 31 sampling sites, 18 were located within Phra Lap and Nai Muang Sub-Districts (hereafter, the Phra Lap region) and 13 were situated within Don Chang and Ban Wa Sub-Districts (hereafter, the Don Chang region) (Fig. 1).

Fieldwork was conducted in June 2012, September 2012 and January 2013. Sampling in June was to capture the habitat conditions at the beginning of the rainy season. Sampling in September was to measure the habitat conditions of the wettest period of the rainy season, a time period that also corresponded to the peak in snail reproduction according to Brockelman et al. (1986). Sampling in January was to measure the habitat conditions of the drier and cooler season, a time period reported to have the highest snail densities (Lohachit, 2004–2005). Each site was visited during the three sampling periods. Some sites, however, particularly the rice paddies, were completely dried up due to seasonality and therefore could not be sampled. As such, across all sampling periods, instead of having 93 samples (31 identified sites for three sampling periods), a total of 80 samples were obtained.

2.2. Sample collection

Sample collection at each site included water quality measurement and snail sampling. A hand-held Global Positioning System device was used to record the coordinates of the sites.

2.2.1. Water quality measurement

Two YSI 556 Multiprobe System water quality meters were used to measure water quality variables, including temperature, salinity, electrical conductivity, total dissolved solids (TDS), dissolved oxygen (DO), and pH. These variables have been considered as possible abiotic factors influencing *Bithynia* snail distributions and measured in prior studies of other areas (i.e., Lohachit, 2004–2005; Ngern-klun et al., 2006). The meters were submerged into water for 10 min to allow the readings to stabilize before recording. In addition, because *O. viverrini* eggs from human and animal feces washed into the freshwater environments are the main source of snail infection, a 5-ml water sample was collected per site and poured into a Coliscan Easygel testing kit to measure the level of *Escherichia coli* (*E. coli*) contamination. The Colisan Easygel kits were kept on ice during the field work and then transported back to the laboratory for analysis.

2.2.2. Snail sampling

Various methods have been employed for snail sampling, including collection within a fixed time period (e.g., Suwannatrai et al., 2011), the Ekman dredge method (e.g., Haruay et al., 2008), and quadrat sampling (e.g., Lohachit, 2004–2005). To enable quantification and comparison of snail abundance and diversity across different sites and in consideration of the site conditions for sampling, a quadrat sampling (i.e., Lohachit, 2004–2005) was adapted for snail collection in this study. Along the shallow edge of the water body of each sampling site, a 0.5-m square quadrat measuring 0.25 m^2 in area was placed four times at every meter interval along a transect to measure a total quadrat area of 1 m^2. All snail species found within the quadrat were collected for subsequent analyses.

2.3. Sample processing and analysis

2.3.1. Water samples for estimating fecal contamination

The level of *E. coli* in the water of each sample site was derived according to manufacturer instructions of the Coliscan Easygel

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